

The Journal of the American Association of Zoo Keepers, Inc.

Animal Keepers' Forum

A close-up photograph of a squirrel being bottle-fed. A person's hand is visible, holding a small white plastic bottle with a red cap. The squirrel is looking directly at the camera with large, dark eyes. Its fur is brown and white. The background is blurred green foliage.

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American Association of Zoo Keepers, Inc.

The American Association of Zoo Keepers, Inc. exists to advance excellence in the animal keeping profession, foster effective communication beneficial to animal care, support deserving conservation projects, and promote the preservation of our natural resources and animal life.

ABOUT THE COVER

This month's cover comes to us from Mari Belko of Phoenix Zoo and features a cownose ray (*Rhinoptera bonasus*). Cownose rays are related to sharks and skates. This stingray belongs to the Family *Myliobatidae*, which includes bat rays, manta rays and eagle rays. Cownose rays get their name from their unique forehead, which resembles the nose of a cow. They are brown to olive-colored on top with no spots, and pale below. Beach-goers sometimes mistake these rays for sharks. When the rays are swimming near the surface, the tips of the wings sometimes stick out of the water, resembling a shark's dorsal fin.

Cownose rays can be found in the Atlantic Ocean along western Africa, the eastern U.S., the Gulf of Mexico and parts of the Caribbean. They are considered an open ocean species, but can inhabit inshore, shallow bays and estuaries. They prefer warm temperate and tropical waters to depths of 72 feet. Cownose rays feed on bottom-dwelling shellfish, lobster, crabs and fish. To locate their prey, cownose rays have electroreceptors on their snouts as well as excellent senses of smell and touch. They will stir up the bottom with their flexible wing tips or use their noses to root around in the mud or sand. Once they find their prey, they flap their wings rapidly to move the sand aside.

Articles sent to **Animal Keepers' Forum** will be reviewed by the editorial staff for publication. Articles of a research or technical nature will be submitted to one or more of the zoo professionals who serve as referees for **AKF**. No commitment is made to the author, but an effort will be made to publish articles as soon as possible. Lengthy articles may be separated into monthly installments at the discretion of the Editor. The Editor reserves the right to edit material without consultation unless approval is requested in writing by the author. Materials submitted will not be returned unless accompanied by a stamped, self-addressed, appropriately-sized envelope. Telephone, fax or e-mail contributions of late-breaking news or last-minute insertions are accepted as space allows. Phone (330) 483-1104; FAX (330) 483-1444; e-mail is shane.good@aazk.org. If you have questions about submission guidelines, please contact the Editor. Submission guidelines are also found at: aazk.org/akf-submission-guidelines/.

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FROM THE BOARD OF DIRECTORS



"AAZK strives to serve as a resource for its members to participate in conservation programs and activities, and hopes to continue to collaborate with our institutional partners to offer keepers conservation opportunities worldwide."

Since 1967, the American Association of Zoo Keepers (AAZK) has served as a professional resource for its members, regardless of job title, within the field of animal care. Over the years, our role as zoo keepers, aquarists, and vet techs has evolved. We are no longer solely responsible for scooping poop and preparing diets. The ever increasing roles zoo and aquarium professionals play in *in situ* and *ex situ* conservation initiatives emphasizes the growing importance of AAZK's conservation programs and the opportunities they provide our members and Conservation Partners.

As Board Oversight for the Conservation teams, I work closely with the Conservation Committee, the Trees for You and Me (TFYM) Program and the Bowling for Rhinos (BFR) Program. These teams strive to provide resources for keepers to collaborate and participate in conservation projects both at their zoos and aquariums and beyond. As zoos and aquariums continue to develop these conservation programs, AAZK aims to bridge the gap between keepers and conservation. AAZK strives to serve as a resource for its members to participate in conservation programs and activities, and hopes to continue to collaborate with our institutional partners to offer keepers conservation opportunities worldwide.

This is the second year of the newly restructured TFYM and BFR Programs, and they are quickly growing. TFYM has recently completed an event kit to make it even easier for AAZK Chapters to host events to raise awareness of the degradation of arctic habitats and how that threatens not only polar bears but countless other species. The TFYM Grant (free money!) is also unique in the fact that 100% of the money raised through this program goes directly to fund reforestation and habitat revitalization projects. The TFYM Program raised over \$18,000 in 2017, and is on track to exceed this total in 2018.

Bowling for Rhinos, AAZK's flagship fundraiser, had another immensely successful year in 2017 raising \$607,580.69 for rhino conservation. This success is attributed to the dedication of our members who have been hosting bowling and other themed events since 1990, resulting in a grand total of \$7,220,876.93 raised! The BFR Program has been working to assist Chapters to develop and improve their events, as well as connect our BFR Conservation Partners, Lewa Wildlife Conservancy, International Rhino Foundation and Action for Cheetahs in Kenya, with our membership.

The Conservation Committee has been expanding the Conservation Partner program, allowing AAZK to foster cooperation and partnership with organizations striving to preserve species and their natural environments around the world. This year, we have introduced a new Conservation Partner category: Conservation Partner (Member Association), which includes some outstanding organizations such as Animal Behavior Management Alliance (ABMA) and Association of Zoological Horticulture (AZH). The Conservation Committee's goal is to help AAZK membership connect with, and participate in, conservation projects globally. The Conservation Opportunity Database, located on the Conservation Committee's page on AAZK.org, includes organizations accepting volunteers to participate in conservation efforts around the globe.

This has been an exciting journey for me: meeting committee members, learning how the teams function, and being inspired by each individual's passions. I look forward to watching the Conservation team grow and continuously build upon AAZK's mission to support deserving conservation projects and promote the preservation of our natural resources and animal life.

A handwritten signature in black ink that reads "Azzara Oston".

Azzara Oston

Azzara.Oston@AAZK.org

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COMING EVENTS

Post upcoming events here!
e-mail shane.good@aazk.org

July 22-28, 2018

Felid TAG Meeting and Husbandry Course
Fresno, CA
Hosted by the Fresno Chaffee Zoo
For more information go to:
<https://www.facebook.com/felidtag/>

August 12-15, 2018

2018 Symposium on the Conservation and Biology of Tortoises and Freshwater Turtles
Fort Worth, TX
For more information go to:
turtlesurvival.org/conference#WqaSnqJld-Y

August 23-25, 2018

International Symposium on Pangolin Care and Conservation
Brookfield, IL
Hosted by Chicago Zoological Society
For more information contact:
amy.roberts@czs.org

August 26-29, 2018

Association of Zoo Veterinary Technicians
Columbus, OH
Hosted by Columbus Zoo and Aquarium
For more information go to:
azvt.org

September 17-28, 2018

Smithsonian-Mason School of Conservation. Ecology and Conservation of Migrating Birds
Front Royal, VA
For more information go to:
smconservation.gmu.edu

September 23-27, 2018

AZA Annual Conference
Seattle, WA
Hosted by Seattle Aquarium and Woodland Park Zoo
For more information go to:
aza.org/conferences-meetings#mym

October 8-12, 2018

From Good Care to Great Welfare
Detroit, MI
Hosted by The Detroit Zoological Society's Center for Zoo and Aquarium Animal Welfare and Ethics
For more information go to:
www.czaw.org/events

October 14-18, 2018

International Congress on Zookeeping
Buenos Aires, Argentina
Hosted by Fundacion Temaiken and the International Congress of Zookeepers
For more information go to:
iczoo.org/congress

October 15-20, 2018

Otter Keeper Workshop
Portland, OR
Hosted by Oregon Zoo
For more information go to:
otterkeeperworkshop.org/



October 4-8, 2018

AAZK National Conference
Denver, CO

Hosted by the Rocky Mountain AAZK Chapter and Denver Zoo

rmaazk.org/2018-national-aazk-conference/

October 25-27, 2018

Waterfowl Conservation Workshop
Greenville, NC
Hosted by International Wild Waterfowl Association and Sylvan Heights Bird Park
For more information go to:
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AAZK Resource Committee

Aquaculture Supporting Conservation

Ramon Villaverde, Senior Aquarist
Columbus Zoo and Aquarium
Columbus, Ohio

Raising animals has always been an interest of mine, even before I started my professional career as an Aquarist over 20 years ago. As a hobbyist in college, I bred several species of freshwater fish at home that I would sell to a local fish store where I worked to pay for my hobby. At the time, I just thought it was a cool opportunity. When I started my career, I didn't realize the

importance of aquaculture work within our field and for conservation efforts.

Marine aquatic animal husbandry can be challenging. There are so many things that an aquarist needs to understand to keep our animals alive. How big do the animals get? Will they eat other animals? Are they aggressive with each other? What kind of

water chemistry do they require? Those are just a few questions in a long list, but one sign that you are doing something right is seeing them reproduce.

There are several ways that marine fish reproduce. There are mouthbrooders, benthic spawners and pelagic/broadcast spawners, with the two latter methods being the most common. Most facilities don't put any effort into rearing the eggs, other than for a few benthic marine species like clownfish, due to the time and difficulty to do so. This, however, began to change when the RisingTide Conservation Project started in 2010. The project was funded through an Association of Zoos and Aquariums Conservation Endowment Fund (AZA CEF) Grant. The goal of the project was to:

1. Develop and perfect techniques for collection of marine tropical fish eggs
2. Perfect techniques of shipping eggs to other facilities
3. Determine appropriate foods for larval rearing
4. Develop sustainable food production for larval feeding
5. Develop best methods for larval rearing

Our role in the project was to help develop the techniques for egg collection and shipment of the eggs to University of Florida Tropical Aquaculture Laboratory (TAL) in

Angelfish at 100+ days post-hatch.





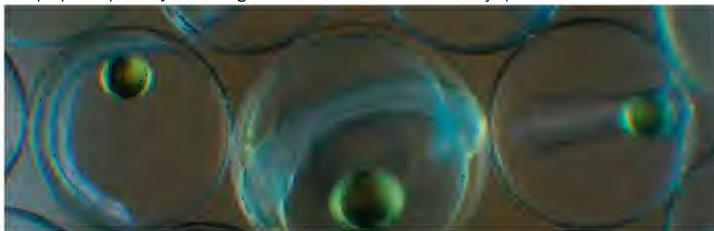
Two-liter bottles used as starter algae cultures.



The grey container in the aquarium is our DIY egg collector.



A re-purposed pretzel jar with angelfish larvae that are about 25 days post hatch.



A mass of eggs from our 85,000 gallon mixed Indo-Pacific system.

Florida. After a few trials and errors, our developed methods help TAL produce several species of fish from the Columbus Zoo and Aquarium as well as other AZA facilities involved with the project.

Around the same time I started to tinker with the eggs we collected as well. I didn't have the same infrastructure as TAL for aquaculture, but was able to set up a live foods and larval culture area repurposing water jugs, bottles and buckets. The partnership with TAL also supplied me with my starter cultures of algae and copepods. The first success was raising a few angelfish, *Pomacanthus* sp., from our 85,000 gallon mixed Indo-Pacific exhibit. Another project started a few years later by Roger Williams University, Institute of Museum and Library Services and New England Aquarium. The goal was to close the loop on exhibit species and provide the basic tools to facilities to do it themselves. Every institution involved was given basic training on live foods and larval rearing at several workshops held at Roger Williams University. Participating facilities were also given a larval rearing starter kit. This has really boosted rearing efforts around the U.S. The number of new species keeps growing. Research facilities like TAL and The Oceanic Institute have had species first success with pacific blue tang (*Paracanththus hepatus*), and yellow tang (*Zebrasoma flavescens*), respectively. Todd Gardner, a well-known fish breeder, has successfully bred the embargoed Cuban Basslet (*Gramma dejongi*). To date, I have been fortunate enough to successfully aquaculture seven species of fish and six species of invertebrates here at the Columbus Zoo and Aquarium. The knowledge I have gained has been shared at several hobbyist and professional conferences. Animals I have raised have been placed on exhibit at my facility as well as at several aquariums around the country.

All of the recent efforts have had several benefits to our industry. It has shown that there is potential for decreased dependency on wild caught animals for public exhibits. Animals that have been raised not only have enhanced exhibits, they have also become ambassadors for the industry. Additionally, these animals help connect our efforts with issues that our natural environments are facing. I have hopes that with increased support and collaboration, we will rely less on wild caught animals and that these efforts will assist with conserving rare and endangered animals. 

Autophagy in a Non-senescent Giant Pacific Octopus (*Enteroctopus dofleini*) and its Mitigation via Behavioral and Environmental Measures

Adam Egut, Wild Animal Keeper I
Brenna Romig, Wild Animal Keeper I
Akron Zoological Park, Akron, OH



Autophagy, autosarcophagy, and autocannibalism are all terms used to describe the practice of eating oneself (Budelmann, 1998). This practice is not uncommon amongst male giant Pacific octopuses (GPO's) during senescence, or at the end of their short lifespans, which are only about three to five years (AITAG, 2014). However, when a non-senescent, 1.5-2-year-old (estimated) male GPO exhibits such behavior, it's definitely a cause for concern.

When we first noticed large chunks of tissue missing from numerous arms of our GPO, autophagy was not our first theory. See Figures 1 and 2. We first searched around his exhibit for any previously unseen sharp edges or pieces of plumbing that had possibly come loose. We inspected his enrichment items for the same. We took into consideration that some of his food items, mainly crab, had sharp pieces and claws on them. However, none of those things seemed to make sense. There were no sharp edges in his exhibit, no jagged pieces of enrichment, no plumbing knocked loose, and crab is the main staple of any GPO diet. His wounds persisted and grew in number over the course of a few weeks. The gouges ranged in size from only a few millimeters to three or four centimeters in diameter. A second, short-lived, hypothesis was accidental tears similar to how GPO's frequently rip the edges of their suction cups when sloughing off the outer epidermis, much like humans shedding dead skin. The chunks out of his arms just seemed too deep and too numerous to be accidental tears. It wasn't until we caught him arm-in-beak that we knew what we were dealing with.

From back-of-house, the only way to see into the GPO's tank is from above. The sides and back of his acrylic exhibit are covered from the background inside of it. One day, upon stepping onto the platform and removing the lid from his tank, we found our GPO hanging onto the ledge at the top of his rocky cave background, upside down, beak visible, and arm in beak. It was then we had confirmation that those chunks were self-inflicted. Extremely concerned for our GPO's physical and emotional health, we performed copious amounts of research and worked closely with our Aquatics Specialist and Wild Animal Keeper III, Steve Balogh, as well as the Animal Curator and Director of Animal Operations, Pete Mohan. Our GPO was not exhibiting any signs of senescence. No hollow/sunken eyes, lack of appetite, weight loss, uncoordinated movements, or sloughing of necrotic tissues (AITAG, 2014). There were also no spermatophores seen in the tank prior to or after the behavior started. This led us to the conclusion that the behavior was more than likely caused from a stressor rather than senescence.

Extremely concerned for our GPO's physical and emotional health, we performed copious amounts of research...

We sought to mitigate and altogether extinguish this autophagic behavior via the factors which we found in our research to be the most prominent causes in other known cases of stress-related (as opposed to senescence-related) self-cannibalism. No small feat, as the majority of cases of autophagy involve entire arms being eaten and are terminal within just a few weeks of the onset of the behavior (Budelmann, 1998). According to the AZA Aquatic Invertebrate Taxon Advisory Group (AITAG), "Modifying tank enclosures, water flow, or introducing objects create opportunities for animals to make choices and explore environmental changes". We used this



Figure 1. One of the wounds on arm L1.

information along with other findings to decide which factors should be altered in order to curb the behavior.

The factors we evaluated were:

- 1) Water Quality
- 2) Environmental and Behavioral Enrichment
- 3) Diet

1) Water Quality

The exhibit tank capacity is 626 gallons, but taking into consideration the displacement due to the background and substrate, the capacity is likely closer to 575 gallons, while the system capacity is an estimated 1,785 gallons. We also have our Pacific sea nettle tank and our chain catshark tank on this system. We performed every test at our disposal in addition to the daily temperature, salinity, and pH tests and routine ammonia, nitrite, and nitrate tests. The dissolved oxygen (DO) content read at 93% saturation using a YSI ProODO meter. No detectable traces of copper were found using an API Copper Test Kit, and all of the water we use for seawater makeup is passed through carbon and reverse osmosis filters to remove heavy metals. The calcium content was measured at 460 mg/l using an API Calcium Test Kit. We also looked at our records from May 2017, when the animal arrived at our zoo, through November 2017, when the behavior began. The system was kept at an average of 54 degrees Fahrenheit (12.2 degrees Celsius) (n=214), salinity at an average of 32 ppt (parts per thousand) (n=214), and pH at an average of 8.15(n=214). On average no ammonia (n=134) or nitrites (n=155) were consistently read over this same time period using API Ammonia and API Nitrite Test Kits respectively. All of these water quality parameters are in line with the recommended ranges in the AZA Care Manual (AITAG, 2014). Nitrate levels, over the same time period were thought to be within the recommended range of 0-19 mg/l, but we discovered a sample processing error that had been occurring that was resulting in false low nitrate readings. After the issue was corrected, we performed several significant water changes and continued to test nitrate levels daily using API 5 in 1 Test Strips, an API Nitrate Test Kit, and a Salifert Nitrate Profi Test to ensure we were getting accurate readings. Factoring in the false low readings resulted in an actual 7 month average nitrate level of 24 mg/l (n=37). Nitrate levels in the system have been kept within parameters since the testing error was discovered. Additional carbon was placed into the system via a bag in the filter sock in the sump to ensure that no residual harmful pollutants were present in the system from the



Figure 2. One of the wounds on arm L2.



Figure 3. A sample of the enrichment items given to our GPO.

construction of the LSS or the background. The current in the tank was increased via a bulkhead fitting with a PVC nozzle placed in the front right corner, which then jettied water out, down, and up increasing the overall current and rotation of water into and out of the tank. With the exception of the elevated nitrate levels, we could not find any issues with our water quality that would be a possible cause for autophagic behavior. It is also of note that we noticed no health issues with the other invertebrates in the system such as the ochre sea stars or white-spotted rose anemone in our GPO tank or the Pacific sea nettles that share the same LSS.

2) Environmental and Behavioral Enrichment

Being highly intelligent animals, GPO's require appropriate enrichment in order to remain mentally and physically healthy. This is especially important as enrichment has been shown to decrease undesirable behaviors, including autophagy (AITAG, 2014). Our GPO tank was already considered environmentally enriching- from the naturalistic rocky cave background, to the rock floor accented with large stones, to our GPO's tank mates- four ochre sea stars and one white-spotted rose anemone. With the addition of the increased water current, the new bulkhead fitting was installed to allow for interchangeable nozzles that would create different enriching effects in the water: small bubbles, a fast stream, or a

Figure 4. Wound on arm L1 that is showing signs of healing.



wide sprayer. We then focused on the behavioral enrichment our GPO was receiving by increasing the number, variety, and type of enrichment items he was given every day and always being mindful to try to use them in unique, novel ways (See Figure 3). We made sure to interact with him for as long as he seemed to want to interact with us; as he was constantly seeking out human interaction before the occurrence of this behavior by taking tools when you were trying to work, blocking your view of the rest of the tank with his body, and climbing up your arms with his arms. Before his autophagic behavior he would have been described as an interactive, curious, and all-around gregarious octopus. After this self-destructive behavior began he was reclusive and generally uninterested in human interaction. With our additions to his behavioral enrichment we hoped to see that convivial personality again.

3) Diet

Octopuses do not store fat and have high conversion efficiency of feed thus converting food energy into rapid growth which makes overfeeding of little concern (AITAG, 2014). The AZA Giant Pacific Octopus Care Manual goes on to say that feeding octopuses to satiation is an acceptable feeding strategy, but comes with caveats (AITAG, 2014). Some of these caveats include compromising water quality due to high amounts of nutrient inputs, as well as the GPO increasing in size consistent with the amount of food supplied and not with the size of tank allotted (AITAG, 2014). It's important to take into account the growth rate and health of the animal along with the water chemistry and size of enclosure (AITAG, 2014). The feeding schedule we were following included two to three fasting days per week to ensure that our GPO would not outgrow his enclosure. When confronted with autophagic behavior, we thought it better to avoid the possible stressor of non-daily food availability and began feeding our GPO every day. We also increased the variation in his diet items: crab, squid, mussels, shrimp, and herring as well as the mode of delivery: either given to him directly, through enrichment items, or within "octo-pops" (saltwater and food popsicles made especially for him).

Conclusions

While it is theorized that autophagy may be associated with possible viral or bacterial infections (Budelmann, 1998; Seeley et al., 2016), there was no evidence of any infectious disease occurring with our GPO.

Since implementing the changes in enrichment and feeding frequency, as well as increasing the water current in the tank,

we have not seen any additional wounds nor have we caught him "chewing" on his arms. Some of the larger chunks of tissue have yet to completely heal and he often keeps those parts of his arms tucked inward with his suction cups touching so we aren't always able to get a good look at them. When he does allow us to outstretch his arms and evaluate their appearance it is clear that healing is taking place. There are distinctly new epithelial cells closing in on some of the wounds with chromatophores intact (See Figure 4). While one study saw approximately seventy percent re-epithelialization after only eighteen hours following a complete arm tip removal in the common octopus (*Octopus vulgaris*), we have only seen approximately fifty percent wound closure after about four months, with some wounds healing more and some less or not at all (Shaw et al., 2016). In the same study, almost complete wound closure in some individuals occurred within six to twenty-four hours (Shaw et al., 2016). Although his healing has been somewhat slower, he is healing and we have noticed an increase in his activity level, interest in enrichment, and a return to some of his more mischievous behavior. While we could not determine the exact cause of the autophagy in this animal, we were able to use behavioral and environmental measures to reduce or eliminate the behavior and give our GPO a longer, more enriched life.

While the impact of elevated nitrate levels on this issue was unknown, it would be valuable for others experiencing autophagy to see if there is any correlation between the two.

Contact Information

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The Lone Wolf: Early Stages of Wolf Eel Target Training

Shelly Pettit, Sr. Keeper Herps/Fish
Oregon Zoo
Portland, Oregon

The Arrival

The Oregon Zoo received our wolf eel (*Anarrhichthys ocellatus*) in February of 2015 from a nearby aquarium facility that was closing its doors. We received other animals in the same shipment, but I will never forget the day that "Blanche" arrived. At the time Blanche was small, scared, and like every animal, went into a quarantine holding tank where she would spend at least the next thirty days. I gave her plenty of cover, PVC hiding pipes, and even floated fake kelp at the surface so she would feel safe. I offered that animal every type of food imaginable from the typical shrimp, capelin, squid, and herring to sending out the nutrition center staff in search of live goodies like crab, urchins, and spotted prawns. All of these prey items just ended up as tank mates with Blanche because she wasn't interested in eating anything! Being an aquarist I know this isn't uncommon and although the veterinarians were reluctant to let her leave quarantine without visual confirmation of eating, I assured them that her going on exhibit and getting settled in her

new home would be our best bet at getting her finally comfortable enough to eat. They agreed, and in she went.

The First Supper

Our Kelp Forest exhibit is about 23 feet deep and a total of 19,000 gallons. It houses a variety of rockfish, perch, sea anemones, and stars and now a lone wolf eel. At this time, the only way to offer the animal food was by a SCUBA diver. For almost six weeks that animal continued to refuse food and on week twelve I remember cramming every type of food (again) into my white mesh bag and getting ready to take it to the bottom of the tank with my fingers crossed. The stars and planets must have been aligned that day because finally! Finally, Blanche came out and took a piece of clam and a capelin and filled that empty belly. I was overjoyed of course, because as we all know in this field, small victories lead to bigger and better things. Divers came up, we all high-fived, the entry went into ZIMS and a burden had been lifted. For now.

Early stages of introducing the target in feed sessions.

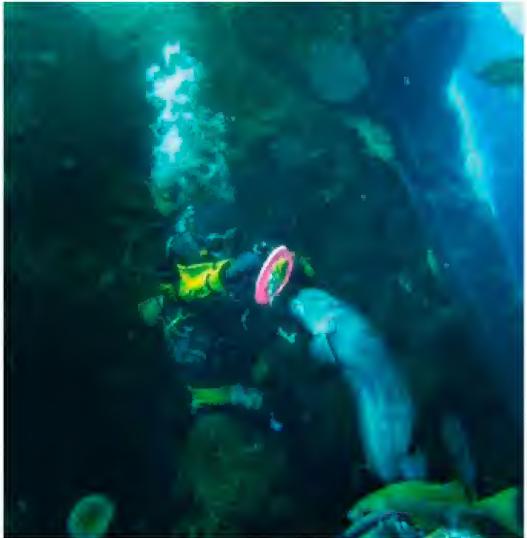


On Exhibit

Diving is a task that occurs every day for a multitude of reasons at our facility. Divers entered the water once a week to feed Blanche, and with a small dive program and a handful of volunteers, we couldn't just be diving one tank all the time. Seals and otters needed a clean pool too. During one dive, specifically for a window clean and a vacuum, Blanche had keyed in on a diver whose dry suit booties happened to have a yellowish sole. We believe that not only was Blanche associating divers with food but it seems this particular dry suit boot also gave her the impression she was looking at her white mesh food bag. Blanche came out in a hungry hurry and gave the bootie a little bite. Nothing hard, nothing to cause injury, but enough to make for a good story. Of course, throughout the day, the stories of Blanche and her "attack on the diver" grew and grew and I realized that it was time to implement some feed training so people wouldn't be afraid to dive with the "ferocious wolf eel that liked to eat feet."

Target Tenacity

Choosing a target can be harder than some think. I traveled the zoo in search of the perfect target. From the lion house to the otter



Wolf Eel continues to learn to "hit the target".

shelves I searched and searched. Finally, one day while browsing the primate enrichment I came across a stack of Frisbees and the idea just hit me. Since us humans are only graced with two hands, I needed to be able to hold the food bag, the target, and reward the animal all at the same time. So what if, the target and the food were being held at the same time? What if Blanche had to grab the food while it was, in theory, attached to the target? I grabbed a bright pink Frisbee and got to work. I cut out the center of the Frisbee, leaving a ring with a large enough diameter that Blanche could get her head all the way through.

And So It Begins

It started out as most target training does. The target was presented only during feed dives and the food item (typically clam as it is her favorite) was held directly in the center of the target. I was surprised at how quickly Blanche caught on. She would swim right up, grab the food, eat right there in front of the diver and wait for the next piece to make its appearance. As I mentioned, dives would occur in that exhibit that were not feed specific, and soon after target training started, Blanche would only appear when she saw the bright pink ring, otherwise she would just watch the divers from her hidey-hole. Dive booties were finally off the menu.

An Evolution in More Ways than One

As target training evolved so did Blanche. Over the next year or so if two divers were in, we would have two targets. One target would be presented at a time and once food was offered, Blanche would be encouraged to turn around and see the other diver and the target. Soon we had that animal swimming all over the tank and hitting her target like a champ. In addition, Blanche also developed an outstanding amount of patience and comfort level with me and the other divers that I had not expected. For example, sometimes, getting the food item out of the bag while wearing those bulky gloves can be troublesome. However, Blanche would hover patiently in front of the target and wait, very still, and very calm, for the next piece of food to arrive target center. She will now also

allow you to touch, hold, and/or cradle her during feed sessions, ultimately making her my favorite animal in the collection. During the initial training and due to the incident with our volunteer, it was decided that only trained staff, typically myself and another person, were allowed to feed Blanche or even dive in Kelp until I was fully confident in her ability to differentiate between feed dives and maintenance dives. After a year of spending so much time with Blanche and being so concerned with her training and well-being a giant transformation had occurred and I didn't really notice until one day while watching a feed. Blanche had come out to eat and the

Fish really are spectacular animals and can be taught a variety of things if we just give them the opportunity to learn.

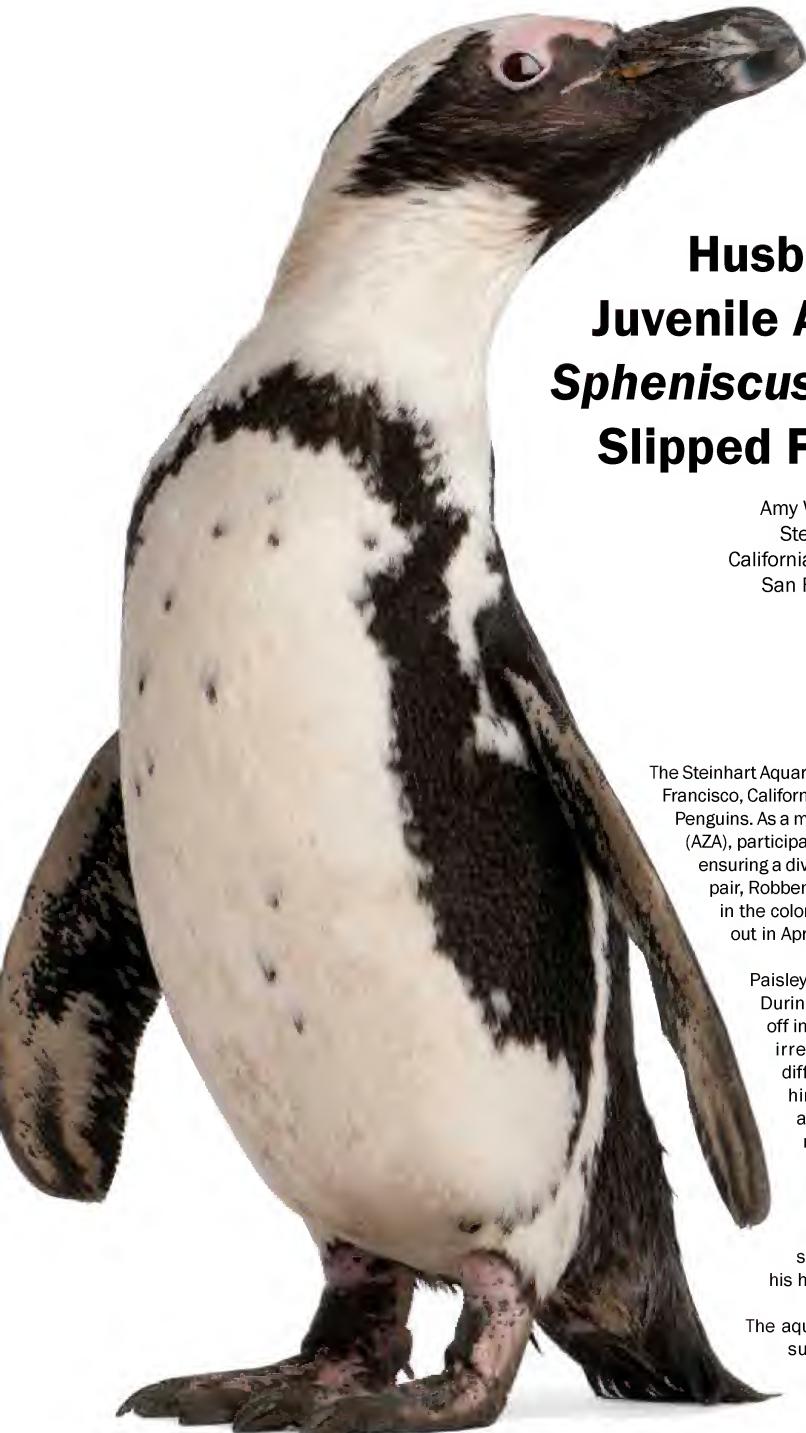
diver at the time had gotten her quite a ways up that tank, at least 10-12 feet into the water column. I noticed how large Blanche had gotten, and quite frankly what a beautiful animal she had become. Quite the transformation from the scared, pale, little eel that had arrived over a year earlier. This realization had led me to the biggest evolution of all. Blanche was not a female, but in fact, a male, and a fine specimen at that. Without the training and giving Blanche the opportunity to form a bond with divers I don't know when I would have figured this out. I only bring this up because, let's be honest, it is kind of funny, and I also think we all have an animal story that we can relate this too. It's been hard to refer to "her" as "he" and retrain everyone to do so but we are working on it.

The Future

While target training continues, my ideas for the future of his training do too. We plan on adding an actual female to the tank soon and that should make for some great opportunities for dual training. We have plans on showcasing this gentle giant for public feeds and special events thanks to his uncanny ability to "wow" people. Fish really are spectacular animals and can be taught a variety of things if we just give them the opportunity to learn. I think we can really open the doors to progress, ingenuity, and animal welfare for our finned friends by sharing our stories and using each other as resources. I've already seen some amazing things that my fellow aquarists have done and I am proud to be in a field with such a great group of folks who always put their animals first and show people what today's zoos and aquariums are all about. 

Close up of the "Lone Wolf Eel".





Husbandry Care for Juvenile African Penguin, *Spheniscus demersus*, with Slipped Flexor Tendons

Amy Walters, Biologist II

Steinhart Aquarium

California Academy of Sciences

San Francisco, California

The Steinhart Aquarium at the California Academy of Sciences in San Francisco, California, houses a colony of 14-20 endangered African Penguins. As a member of the Association of Zoos and Aquariums (AZA), participating in the Species Survival Plan (SSP) is key for ensuring a diverse genetic pool for the captive population. One pair, Robben and Ty, have produced the majority of offspring in the colony in recent years. Their last two chicks hatched out in April 2016.

Paisley, the Beta chick, hatched out 28 April 2016. During incubation, his egg was sometimes pushed off into the corner of the nest, resulting in incubation irregularities that led to suspected hatching difficulties. Staff assisted in the hatch, removing him from his shell, with his umbilicus not fully absorbed (see Photo 1). In the nest, Paisley was neglected by his parents, so he was hand-reared by staff. In August, at four months of age, Paisley and his brother, Prince, were intended to move onto exhibit with the rest of the colony. However, before they were moved, Paisley developed a slight limp. A few days later, he was walking on his hocks, unable to put weight on either of his feet.

The aquarium veterinarian, Dr. Freeland Dunker, DVM, suspected that this change in mobility might be either a neurological or musculoskeletal problem. Two days following standing on his

hocks, Paisley was spending all of his time on his belly using his feet to push himself along the floor. He could not stand upright on his own. He was confined to a smaller penned-off area, still in the same room as Prince for several days so he would not injure himself. Veterinary staff noted that despite being confined, his condition progressed, his hocks became swollen, and the Achilles-like flexor tendons appeared loose in both feet.

To rule out a neurological problem, an MRI of the spine was done at a local facility where he was also evaluated by a veterinary neurologist. The MRI and neurologic exam were normal, leaving a musculoskeletal problem as the culprit. Dr. Dunker decided to vet wrap Paisley's feet and legs to provide him with greater stability when he was walking or standing. This made it possible for him to stand for short periods of time and walk short distances.

On 25 August 2016, Dr. Dunker felt he could easily slip the flexor tendon medially on both legs and they preferred to stay there. The following week, Paisley was brought to The Medical Center for Birds in Oakley, California where Dr. Brian Speer, DVM, DABVP (avian practice), DECZM (avian), and Dr. Dunker surgically stabilized the flexor tendon in both legs preventing them from slipping medially. Within two days, he was able to walk slowly with his hocks off the ground. Swelling was minimal and both tendons felt stable. Within a week, there

was a notable lameness in his left leg along with increased swelling but he was still able to walk. The flexor tendon felt loose but remained in place.

By mid-September, Paisley was struggling to walk and Dr. Dunker discovered that the flexor tendons in both his legs had slipped medially again. Dr. Dunker called Dr. Speer to discuss the case and decided to repeat the surgery. Dr. Dunker performed the surgery on site with his associate Dr. Serena Brenner, MS, DVM, to again stabilize the tendons in both legs preventing them from slipping. The next day Paisley was sitting back on his hocks with his feet up (see Photo 2). In order to provide extra support, a brace was created placing his feet in their proper anatomical position. His posture improved, helping him to stand upright, and both flexor tendons remained in place but felt loose. The team felt encouraged that his posture had improved with additional support.

To facilitate standing, Paisley started to put all of his weight on his hocks and support himself with his tail. The feet themselves were off the ground and splayed out. The Academy's Experience Engineering department developed a prototype for a 3D printed boot he could wear (see Photo 3). This would replace the splint and allow him to swim while still having his feet supported. The boot was applied after his morning swim and removed before the end of the day. However, the design was too bulky and made him uncomfortable. In early October

the flexor tendons in both legs had slipped medially leaving him unable to stand.

A novel surgery was performed by Dr. Speer, Dr. Celia Valverde, DVM, DACVS, Dr. Brenner, and Dr. Dunker using a bone anchoring technique with an orthopedic surgical suture (FiberWire®). This was in an attempt to secure the loose flexor tendons in place to prevent them from slipping medially. The left leg was performed first and the right two weeks later because of the length of surgery and the ability to make any necessary adjustments to the surgical procedure on the right leg. By mid-November, Paisley was able to shuffle walk and swim well. He was often found standing with both feet supporting him. He was standing on his hocks but it was accepted that he might stand like this permanently. His flexor tendons remained in place.

By early December, Paisley was struggling to stand properly and developed a limp on his left side. His left ankle joint had become infected. He was able to walk and stand for short periods of time but fatigued quickly. Surgery was performed to remove the FiberWire® which had become infected. Without the support of the orthopedic suture keeping the flexor tendon in place, it slipped medially. Paisley reverted back to supporting himself with his tail and both feet entirely off the ground. Within a couple weeks, he was unable to support himself at all and was on his belly all day. Paisley was given an hour of swim time daily for

Photo 1. Paisley after he was removed from shell with open umbilicus. Photo by Amy Walters.



Photo 2. Paisley was unable to stand on his feet, supporting himself on his hocks and tail. Photo by Amy Walters.



Photo 3. 3D printed boot for Paisley. Photo by Amy Walters.





Photo 4. A baby bouncer hung in the doorway of the hospital. Photo by Amy Walters.



Photo 5. A small dog harness for Paisley to wear to enable him to push his feet along the floor without applying too much pressure.

mental stimulation and to get weight off his feet. The team noticed he was able to walk for short periods post-swim. Eventually the surgery failed on the right leg and the flexor tendon slipped medially leaving Paisley unable to walk.

The decision was made prior to a potential

Photo 6. 3D model of Paisley's leg bones.
Photo by Amy Walters.



next surgery to bring in a veterinarian with a background in physical therapy to help Paisley build strength in the muscles and tendons in his legs. Dr. Erika Gebhard, DVM, CVA, CCRT, believed that the reason his hocks were dropped was due to a general weakness in his legs. Biologists conducted two to three physical therapy sessions per day. A baby bouncer was brought in to allow Paisley to stand while putting minimal weight on his feet, allowing his feet to flex in the proper position (see Photos 4 and 5). The physical therapy sessions brought a surprising amount of strength back, but he was still unable to stand due to the medial position of the flexor tendons. The hope was that whatever strength was developed in the muscles and collateral ligaments and tendons would benefit him after surgery.

In January, the husbandry and veterinary staff had a meeting to discuss his medical prognosis and more importantly his quality of life. The team decided that Paisley needed to be able to walk properly after the next surgery. The veterinary team would come up with their best surgical plan given what they had learned so far and give the rest of the team a best guess regarding success for walking. The husbandry staff felt Paisley needed to be able to walk well enough to not cause other medical problems and to be able to have an acceptable quality of life.

Paisley made another trip to The Medical Center for Birds in January 2017 for a CT scan to evaluate the anatomy of his leg bones so the veterinary surgical team could devise the best surgical approach

and technique. A 3D model from the CT scan was printed to give the veterinarians a real-size model in order to make sure the size of the instruments and devices that would be used during surgery would work (see Photo 6). The 3D model showed the groove and notches of the bones in the area of the joint where the flexor tendon rests were abnormally shaped. This caused the tendon to slip medially interfering with normal walking mechanics. These bone abnormalities helped explain why the previous surgeries were not successful. The tendon needed to be even more secure in order for the surgery to be successful. A lacing technique was proposed to help better secure the flexor tendon in place in multiple locations, similar to shoe laces, giving what the surgical team felt a reasonable chance for success for walking.

Prior to the surgery on Paisley, the veterinarians practiced the procedure on a cadaver penguin of similar age stored in the Academy's collections. The final surgery was performed on 24 February 2017. Initially, the surgery appeared to be a success, but a week post-surgery the flexor tendons slipped medially again in both legs. Given the quality of life criteria put in place prior, the failure of his final surgery was cause for Paisley to be euthanized. Dr. Speer was consulted as well as Dr. Valverdi and they agreed with the decision. All surgical options had been exhausted at that point and quality of life was the priority. Paisley was euthanized at 11 months of age.

During the necropsy of Paisley's legs it

was discovered that the surgery itself was successful in anchoring the flexor tendon to the bone at the site of tendon slipping, but the bone's grooves and notches were too abnormal to keep the tendon in place below where the tendon was anchored. It is not clear whether this abnormality was from birth or if it was developmental due to the abnormal tendon movement and weight bearing on growing legs over time.

The previous year, a juvenile African Penguin from the same parents, Curry, had a medially slipped tendon in only one leg. The team was able to manage this bird's quality of life well through surgeries and supportive wearable gear. Due to postsurgical infection, the leg was partially amputated leaving a stump. Curry is currently at Pueblo Zoo in Colorado and thriving (see Photo 7). 

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Photo 7. Curry after a fun painting session.
Photo by Amy Walters.



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Conditioning Cownose Rays for Physical Exams and Ultrasounds without Restraint

Mari Belko, Keeper, Stingray Bay, Arizona Center for Nature Conservation/Phoenix Zoo
Kelsey Ray, Keeper, Stingray Bay, Arizona Center for Nature Conservation/Phoenix Zoo
Steve Sharp, Collection Manager, Arizona Center for Nature Conservation/Phoenix Zoo
C. Drew Foster, Animal Curator, Arizona Center for Nature Conservation/Phoenix Zoo

Introduction

Atlantic cownose rays (*Rhinoptera bonasus*) are a relatively common species in touch tanks and exhibits of North American zoos and aquariums. The diamond-shaped Atlantic cownose ray (*Rhinoptera bonasus*; Fig. 1) is a moderately-sized stingray, with a disc wingspan of up to three-and-a-half feet – a measurement greater than its length (Last et al., 2016). Their indented snout resembles a cow's nose giving these rays their common name. There are currently 616 animals housed in 29 institutions across North America

(Zoological Information Management System, 2017) and they continue to grow in popularity. They are hardy animals with gentle dispositions, making them well-suited for display in aquariums and interactive touch tank exhibits. Touch tank exhibits contribute greatly to zoo missions by providing guests with opportunities to form connections to these often-misunderstood animals.

At the time of writing, the Arizona Center for Nature Conservation/Phoenix Zoo is home to 27 cownose rays housed

in a 12,000-gallon touch tank (Fig. 2). These animals are accustomed to hand feeding and acclimated to human touch. Consequently, many of the rays develop bonds with their keepers. Analyzing individual medical needs for a specific ray can be challenging. Our goal was to have individual animals participate in their own medical examination without restraint to reduce stress and improve animal care and welfare. We document our training goals and successes in conducting medical exams and ultrasounds on the animals without restraint.



Fig. 1. The diamond-shaped Atlantic cownose ray (*Rhinoptera bonasus*).

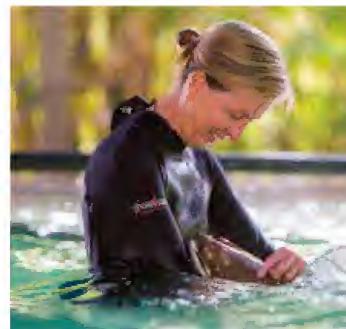




Fig. 4. The keeper stands in chest-high water with forearms submerged and extended forward while offering food.



Fig. 5. Upon approach, forearms were then slid simultaneously under both pectoral fins, careful to not obstruct the gills.



Fig. 6. Each successful positioning was reinforced with food and/or tactile stimulation.

Swim patterns tend to be relatively calm and consistent for zoo-managed cownose rays, but behavior can become erratic when changes occur in their environment. Feeding, mating, competition and perceived threats can affect the animals' behaviors and result in the fever (group of rays) herding together and acting as a collective unit. In managed settings, capture nets are often used to remove animals for medical observation and treatment, and rays display the same erratic behavior when these nets are placed into their environment (pers. obs.). Because of this, we began considering whether this reaction could be reduced or extinguished altogether through alternative strategies including training and the elimination of nets and restraint.

Training Goal

Our goal was to have cownose rays remain stationary in the arms of keepers to allow examinations and ultrasounds without restraint. Examinations can consist of evaluating the ray's eyes, skin, body condition, ventral area, and gill slits. Ultrasounds are a useful diagnostic tool for identifying and monitoring medical issues and pregnancies. The ray must be trained to remain relatively motionless just under the

surface of the water during the examination. Placement of the ray would need to be at the side of the pool to allow medical equipment and staff access. Touch desensitization on the ventral side of the animal is necessary to allow for the ultrasound probe to press against the dorsal side of ray. Ray sensitivity to electrical pulses emitted by the probe in the water also needed to be overcome.

Methods

Training began with two of our largest, more dominant females. These two animals were moved into a separate tank (approx. 10,000 gallons) to keep them in isolation, eliminating potential interference, mating and competition from other rays in the group. This allowed for individual attention between the keeper and ray.

Rays display different levels of socialization to keepers. Some will gently bump their rostrum into the keeper upon face-to-face approach. Others will follow the keeper through the water from behind, bumping repetitively into them. In some cases this action is food motivated but in other cases the ray will refuse food and appears to only be looking for interaction with the keeper. Two of the largest and most dominant rays began swimming up onto the keeper's chest when food was offered (Fig. 3). It was this behavior that became the baseline for our training. We focused on these two rays first since they were in the initial stages of training already.

Fig. 2. (left top) The touch tank exhibit.

Fig. 3. (left bottom) Two of the largest and most dominant rays began swimming up onto the keeper's chest when food was offered.

Training Steps

1. The keeper stood in chest-high water with forearms submerged and extended forward while offering food (Fig. 4). The animal was rewarded upon direct approach.
2. Upon approach, forearms were then slid simultaneously under both pectoral fins, careful to not obstruct the gills (Fig. 5).
3. Forward motion of the animal was maintained for its comfort by having the keeper walking backwards as the ray swam up onto their arms.
4. Time spent stationing on the keeper's forearms increased with each training session as the animal's comfort level increased. Each successful positioning was reinforced with food and/or tactile stimulation (Fig. 6).
5. Rays typically approached the keeper at a 45-degree upward angle (Fig. 7), but for clear medical images to be obtained, the animal would need to be in an orientation horizontal to the water surface. To achieve optimal positioning of the animal, the keeper elevated the posterior portion of the animal upwards while the ray rested on the keeper's forearms. Forward motion by the ray caused the rostrum to press into the keeper's chest, aiding in the animal's stability. Rays



Fig. 7. Typical 45-degree upward angle.



Fig. 8. Placing hands under ventral area.



Fig. 9. Positioning ray close to side of pool.



Fig. 10. The animals were able to overcome challenges for successful ultrasounds.

gradually slowed forward movement submitting to the exam.

This training took approximately three weeks to accomplish, working 15-minute sessions daily.

6. Once proper positioning was established, the keeper discontinued walking backwards. A setback to time spent stationing occurred at this point but after one week in a fixed position the animal became comfortable again and station time intervals increased.
7. When proper stationing was established for 10-second intervals, touch desensitization began. As the ray remained stationed in the arms of the keeper, just under the surface of the water, a second person would run their fingertips down the dorsal aspect of the disc – first down one side and then the other.
- Desensitization happened within three days. As these were touch tank animals, we attribute this short duration to the fact they were already accustomed to human touch. We noticed the animals were more sensitive towards the posterior part of the disc.
8. A simulated probe replaced the fingertip touch with no change in the ray's response.
9. More pressure was added to the dorsal side touch, simulating an ultrasound exam. Additional support for the animal was sometimes necessary on the posterior portion of the ray's disc. The keeper stationing the ray would shift their arms to support the additional pressure placing a hand under the posterior region of the ventral area (Fig. 8).
10. The final phase to training was the introduction of the ultrasound probe into the water. Initially the ray reacted to the use of the probe with restlessness, most likely due to the sensitivity of their electromagnetic sensory system. After approximately three to five attempts with the probe, however, the ray adjusted to the sensation of the new object and submitted to the examination.

Training Challenges

The animals faced a few other challenges that added to the difficulty of the test. The equipment cord was relatively short, so the animal needed to be closer to the side of the pool (Fig. 9). The pressure applied by the veterinarian for the ultrasound was also stronger than the pressure applied in training sessions. Examinations were more successful if the ray was given time to acclimate with the equipment. With repetition and positive reinforcement, the animals were able to overcome these challenges for successful ultrasounds (Fig. 10).

Training Success

The original two animals rejoined the fever in the main exhibit making their training more challenging due to a less controlled environment. However, additional rays have been trained for this behavior in the main exhibit pool following the steps outlined above. So far, eight of the rays can now comfortably station alongside the pool for successful ultrasound examinations without restraint, thus, greatly reducing stress to the animals. Competition amongst animals

We attribute our rapid success in station training Atlantic cownose rays to having a strong and positive relationship with the keeper.

for training can exist so it is helpful to work with two keepers when training rays. One keeper focuses on the training while the other keeper acts as a distraction, deterring other animals from becoming involved in the session.

Discussion

The most important way to train a successful behavior is to focus on the animal and be aware of how they are responding while being prepared to readjust if challenges are encountered. Although we experienced rapid success in training cownose rays for voluntary medical examinations, we did experience challenges. Both keeper and animal adjusted to the training due to these challenges, and keepers always kept training positive with a clear understanding of what was being asked. We attribute our rapid success in station training Atlantic cownose rays to having a strong and positive relationship with the keeper. Another potential factor contributing to

our rapid success was that, as touch tank animals, these rays were already conditioned to touch.

Proper health of any animal is vital. Cownose rays depend upon appropriate water quality, diet and medical treatment. We have demonstrated that training stingrays to voluntarily participate in their medical examinations can reduce stress to the animals by decreasing both the need for restraint and the number of times the animal must be removed from their environment.

Acknowledgements

Numerous Phoenix Zoo staff members contributed to and assisted in this training project and review of the manuscript.

Training Tales

Editorial Comments

By Kim Kezer

Making the time to observe our animals helps keepers get a better understanding of the species and individual animals in our collection. Knowing what is normal, calm and stress-free behavior will help you to quickly realize when the animals are stressed or agitated in the presence of an aversive stimulus, or interactions among the animals due to breeding or aggression as examples. Anytime we can reduce stress when handling an individual or group of animals, it will be a benefit to all involved.

While observing and training animals in the presence of negative stimuli, it is important to understand the concept of thresholds. Animals learn and work best when trained under threshold, meaning the animal is focused, not distracted or concerned about its environment or human interaction. This is the ideal scenario for an animal to learn. You can assess this by the animals' participation, focus and accepting food or secondary reinforcement.

All is going well, but then the animals' behavior quickly changes, becomes nervous, agitated, leaves or won't take reinforcement; these are some indicators that you have "pushed it" too much and have now crossed the threshold to where the animal is unable to learn and becomes more reactionary rather than compliant. Body language and physical indicators are the best way for you to recognize whether or not you are training below or over the animal's threshold.

In this case, desensitizing the ray to accept the probe and tolerate any sensations, working below threshold is critical because this can make the difference between the animal accepting the aversive stimulus or avoiding or becoming uncomfortable with the ultrasound probe. While training, if you see that you have gone over threshold, quickly back off on the stimulus and go to a level below threshold where you know the animal will participate. Plan accordingly for your next training session to avoid crossing threshold.

Over time, thresholds will change during the desensitizing process. By using incremental steps to increase pressure or duration, you will find the correct balance to maintain a positive training session. Training an animal under threshold will promote optimum learning, making training the behavior faster and with far less stress.

Excellent training and thank you for sharing your Training Tale!

References

- Last, P.R., W.T. White, and C.M. Jones. 2016. Rhinopteridae (Cownose rays). Pp. 732-740 in P.R. Last, W.T. White, M.R. de Carvalho, B. Seret, M.F.W. Stehmann & G.J.P. Naylor (eds.), *Rays of the World*. CSIRO Publishing, Clayton South VIC, Australia.

- Zoological Information Management System. 2017. Species holding report for *Rhinoptera bonasus*. Retrieved 22 Dec 2017 from <https://zims.species360.org/Main.aspx>.



We want to hear your Training Tales: the good, the bad and the fabulous!

Did you enjoy the latest Training Tale? Was this information useful or inspiring? Do you have any operant conditioning experiences that others would benefit from reading? Please submit your "Training Tales" and experiences in operant conditioning to share with *Animal Keepers' Forum* readers. This opportunity provides a convenient outlet for you to exhibit your training challenges, methods and milestones with the AAZK member network.

Please submit entries based on the following guidelines:

- a. Submit a brief description of a training project at your facility. These can be 500 words or less, in text or bullet points – it can be longer (up to 3000 words); however, short and simple descriptions with a few images are just as perfect. Details should include the following:
 1. Define the training goal (what did you try to do and for what purpose?)
 2. List important steps (How did you do it – include plans that changed along the way/what worked and what didn't work)
 3. Timeline used (how long did it take)
 4. Tips you learned along the way
- b. Include 3-5 digital photos that clearly depict the animal in the learning process or performing the desired goal (provide photo caption and photographer of each image). Photos need to be 300 dpi and at least 1200 x 1800 pixels.

Please send submissions or questions to:

Kim Kezer at
kkezer@zoonewengland.com
or

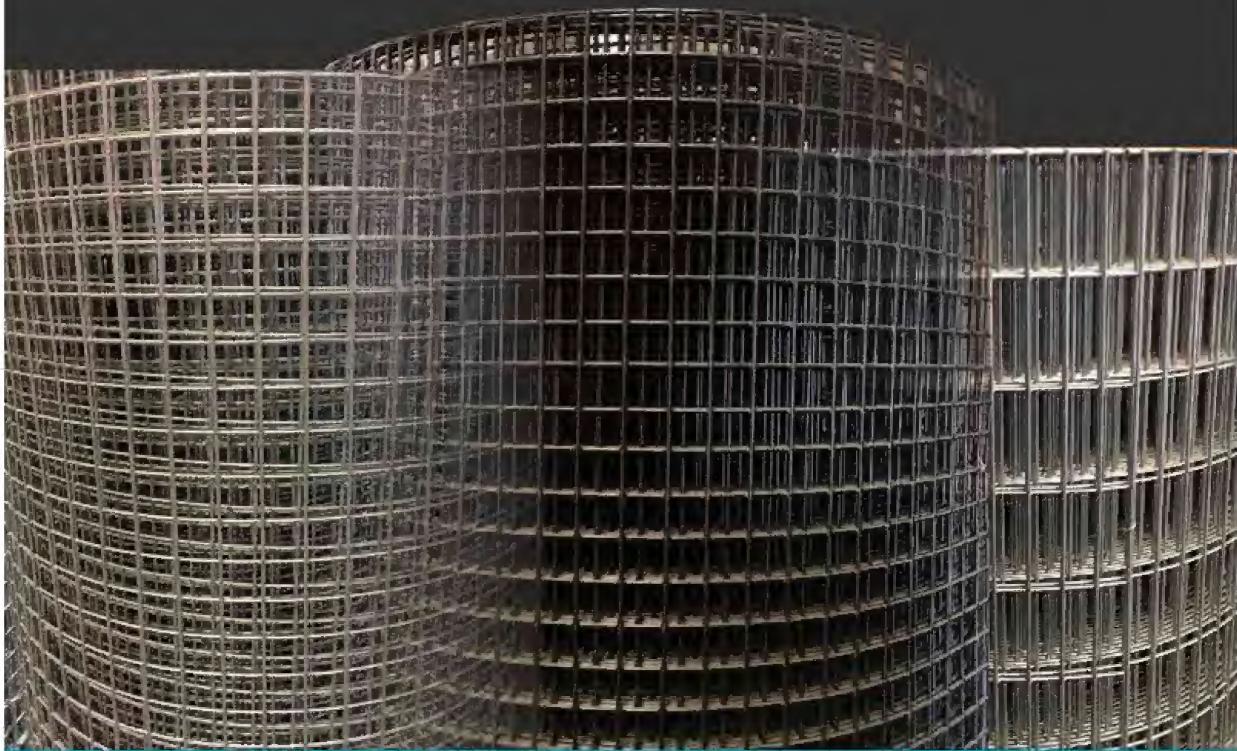
Shane Good at
shane.good@aazk.org
(Use Training Tales Submission as the subject).



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ProCare CCU 48: 52 ¼ W x 33 H x 25 D P/N **912-140**

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